

## Research article

## Open Access

## Characterization of ovine hepatic gene expression profiles in response to *Escherichia coli* lipopolysaccharide using a bovine cDNA microarray

Honghe Cao<sup>1</sup>, Leah C Kabaroff<sup>1</sup>, Qiumei You<sup>1</sup>, Alexander Rodriguez<sup>2</sup>, Herman J Boermans<sup>3</sup> and Niel A Karrow\*<sup>1</sup>

Address: <sup>1</sup>Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada, <sup>2</sup>Department of Clinical Studies, University of Guelph, Guelph, Ontario, N1G 2W1, Canada and <sup>3</sup>Department of Biomedical Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

Email: Honghe Cao - [hcao@uoguelph.ca](mailto:hcao@uoguelph.ca); Leah C Kabaroff - [lkabarof@uoguelph.ca](mailto:lkabarof@uoguelph.ca); Qiumei You - [qyou@uoguelph.ca](mailto:qyou@uoguelph.ca); Alexander Rodriguez - [arodrigu@uoguelph.ca](mailto:arodrigu@uoguelph.ca); Herman J Boermans - [hboerman@uoguelph.ca](mailto:hboerman@uoguelph.ca); Niel A Karrow\* - [nkarrow@uoguelph.ca](mailto:nkarrow@uoguelph.ca)

\* Corresponding author

Published: 29 November 2006

Received: 18 October 2006

BMC Veterinary Research 2006, 2:34 doi:10.1186/1746-6148-2-34

Accepted: 29 November 2006

This article is available from: <http://www.biomedcentral.com/1746-6148/2/34>

© 2006 Cao et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

**Background:** During systemic gram-negative bacterial infections, lipopolysaccharide (LPS) ligation to the hepatic Toll-like receptor-4 complex induces the production of hepatic acute phase proteins that are involved in the host response to infection and limit the associated inflammatory process. Identifying the genes that regulate this hepatic response to LPS in ruminants may provide insight into the pathogenesis of bacterial diseases and eventually facilitate breeding of more disease resistant animals. The objective of this research was to profile the expression of ovine hepatic genes in response to *Escherichia coli* LPS challenge (0, 200, 400 ng/kg) using a bovine cDNA microarray and quantitative real-time PCR (qRT-PCR).

**Results:** Twelve yearling ewes were challenged *iv* with *E. coli* LPS (0, 200, 400 ng/kg) and liver biopsies were collected 4–5 hours post-challenge to assess hepatic gene expression profiles by bovine cDNA microarray and qRT-PCR analyses. The expression of *CD14*, *C3*, *IL12R*, *NRAMP1*, *SOD* and *IGFBP3* genes was down regulated, whereas the expression of *ACTHR*, *IFN $\alpha$ R*, *CD1*, *MCP-1* and *GH* was increased during LPS challenge. With the exception of *C3*, qRT-PCR analysis of 7 of these genes confirmed the microarray results and demonstrated that GAPDH is not a suitable housekeeping gene in LPS challenged sheep.

**Conclusion:** We have identified several potentially important genes by bovine cDNA microarray and qRT-PCR analyses that are differentially expressed during the ovine hepatic response to systemic LPS challenge. Their potential role in regulating the inflammatory response to LPS warrants further investigation.

## Background

The innate immune response to gram-negative bacterial infections is initiated by the recognition of lipopolysaccharide (LPS), a principal component of the cell membrane that is released during bacteriolysis. During systemic infections, LPS ligation to the hepatic Toll-like receptor-4 complex induces the production of a wide variety of hepatic acute phase proteins that are involved in the host response to infection and limit the associated inflammatory process [1]. The secretion of pro-inflammatory cytokines for example, plays an important role in the induction of the febrile and hypothalamic-pituitary-adrenal axis responses to LPS [2,3]. The liver's role in LPS removal and metabolism is also well recognized [4], and likely helps to protect the lungs from acute injury during endotoxemia [5]. Given this, the identification of genes that regulate the hepatic response to LPS in ruminants may provide insight into the pathogenesis of bacterial diseases and eventually facilitate breeding of more disease resistant animals.

A number of studies have previously used microarrays to study hepatic gene expression in rats, mice and dogs challenged with LPS; homologous arrays were used in these studies [6-9]. To date however, only two ruminant microarray studies have been performed with bovine cells stimulated with LPS, and these studies were performed *in vitro* [10,11]. With respect to sheep, ovine microarrays are not currently available. However, two different groups have constructed bovine immune-related cDNA microarrays that hybridize with ovine cDNA [12,13]. These bovine cDNA microarrays may therefore, be useful for assessing ovine hepatic gene expression in response to systemic LPS challenge.

DNA microarray technology is a powerful and frequently used tool for studying differential gene expression. In comparison to quantitative PCR, one of the significant

challenges presented by DNA microarray analysis is having sufficient amounts of high quality RNA that can be labelled and subsequently hybridized onto microarrays. This often requires that animals be euthanized to collect sufficient tissue for RNA extraction, which prohibits the assessment of temporal changes in gene expression *in vivo*. In this study, we amplified total RNA that was isolated from liver biopsy samples and profiled the expression of ovine hepatic genes in response *E. coli* LPS challenge (0, 200, 400 ng/kg) using bovine cDNA microarrays and quantitative real-time PCR (qRT-PCR).

## Results and discussion

### Differentially expressed genes in LPS challenged animals

Gene expression analyses were performed using 8 arrays. Statistical analysis revealed that 11 of genes on the array were differentially expressed between the control and LPS-treated animals ( $p < 0.1$ ) (Table 1). The relative expression of adrenocorticotrophic hormone receptor (*ACTHR*,  $p < 0.07$ ), interferon  $\alpha$  receptor (*IFN $\alpha$ R*,  $p < 0.05$ ), *CD1* ( $p < 0.03$ ), monocyte-chemoattractant protein 1 (*MCP-1*,  $p < 0.04$ ) and growth hormone (*GH*,  $p < 0.04$ ) genes was increased, while complement component-3 (*C3*,  $p < 0.04$ ), myeloid membrane glycoprotein (*CD14*,  $p < 0.10$ ), insulin-like growth factor binding protein-3 (*IGFBP3*,  $p < 0.01$ ), interleukin 12 receptor (*IL12R*,  $p < 0.03$ ), natural resistance-associated macrophage protein-1 (*NRAMP1*,  $p < 0.01$ ) and superoxide dismutase (*SOD*,  $p < 0.08$ ) gene expression was decreased in the LPS-treated animals. Overall, the fold change in gene expression for all of these genes was low ( $\leq 1.49$ ), even though the signal intensity of *MCP-1*, *SOD*, *ACTHR*, *IL12R* and *NRAMP1* was relatively high ( $>5000$  pixels) from the microarray slides.

One of the principle complications in microarray analysis of gene expression is the relatively large amount of RNA required for each array. On average, 5–20  $\mu$ g of total RNA are required per study. This is easily obtained from tissue

**Table 1: Microarray analysis of ovine hepatic gene expression following systemic challenge with 0, 200, or 400 ng/kg LPS**

Gene	Description	p-value	Expression change
<b>200/0</b>			
<i>CD14</i>	Myeloid membrane glycoprotein	0.092	-1.49
<i>ACTHR</i>	Adrenocorticotrophic hormone receptor	0.063	1.05
<i>IFN<math>\alpha</math>R</i>	Interferon receptor $\alpha$	0.047	1.19
<i>C3</i>	Complement component 3	0.038	-1.28
<i>CD1a.b.d</i>	CD1	0.026	1.22
<i>IL12R</i>	Interleukin 12 receptor	0.021	-1.06
<i>NRAMP1</i>	Natural resistance associated macrophage protein	0.002	-1.04
<b>400/0</b>			
<i>C3</i>	Complement component 3	0.090	-1.16
<i>SOD</i>	Superoxide dismutase	0.071	-1.12
<i>MCP-1</i>	Monocyte-chemoattractant protein 1	0.035	1.05
<i>GH</i>	Growth hormone	0.034	1.10
<i>IGFBP3</i>	Insulin-like growth factor binding protein 3	0.007	-1.23

samples collected from euthanized animals, but is more difficult to obtain from small volume biopsy samples collected from live animals. In this study, the SenseAmp kit (Genisphere Inc. Hatfield, PA) was chosen to amplify total RNA. Goff *et al.* [14] evaluated sense-strand mRNA amplification by quantitative real-time PCR analysis. Their results demonstrated that the SenseAmp kit yields the highest correlation between PCR products before and after amplification, and is also able to accurately amplify partially degraded samples.

#### Validated expression of selected genes by quantitative real-time PCR

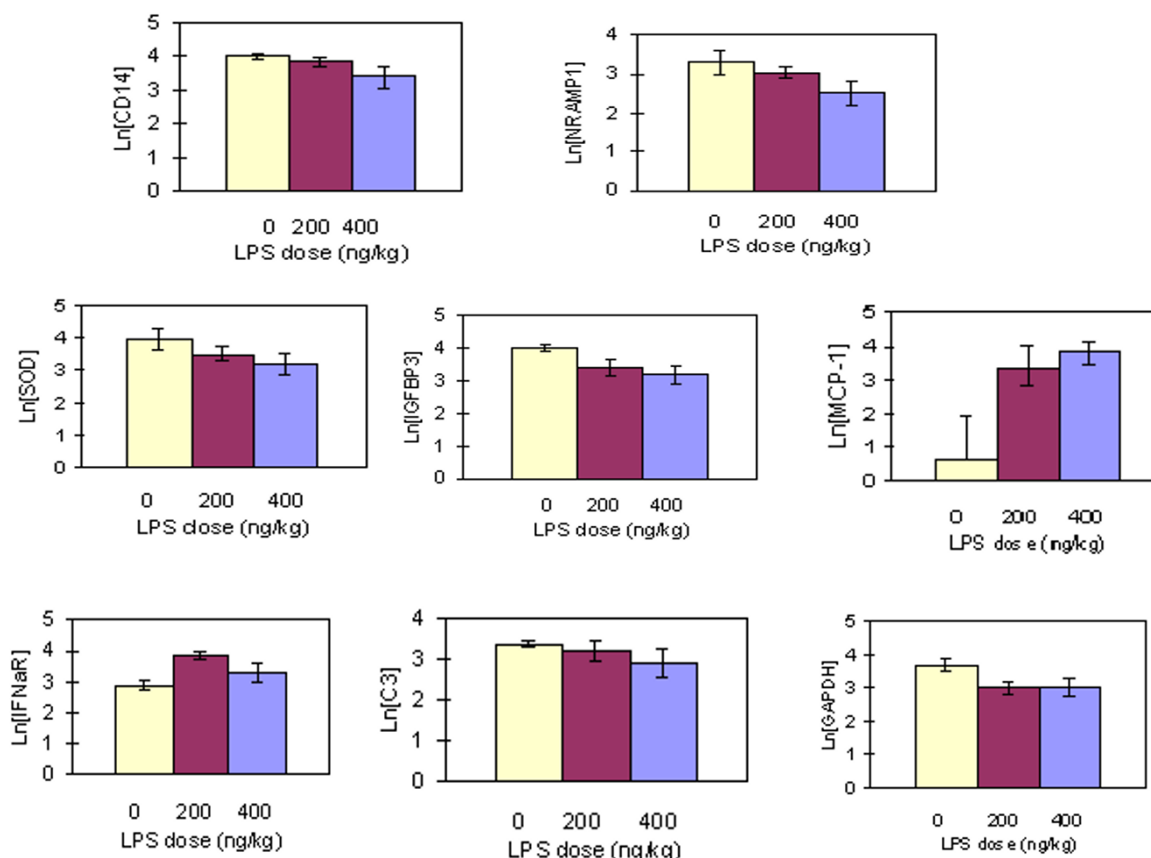
Validation of the microarray results by qRT-PCR was performed on the *CD14*, *IFN $\alpha$ R*, *C3*, *NRAMP1*, *SOD*, *MCP-1*, and *IGFBP3* genes (Table 2). Two housekeeping genes, *GAPDH* and *RPLPO* were also selected. Results from this analysis generally support the microarray data (Figure 1). Linear orthogonal polynomial contrasts (LOPCS) across dose were significant for *CD14* ( $p = 0.06$ ), *NRAMP1* ( $p = 0.05$ ), *SOD* ( $p = 0.07$ ), *IGFBP3* ( $p = 0.03$ ), and the *GAPDH* housekeeping gene ( $p = 0.05$ ), indicating that the expression of these genes was reduced across LPS doses. *GAPDH* has also recently been reported to not be a reliable hepatic housekeeping gene for rats challenged with LPS [9]. LOPCS across doses was also significant for *MCP-1* ( $p = 0.02$ ), indicating that the expression of this gene was increased across LPS doses. A significant quadratic orthogonal polynomial contrast across dose was also noted for *IFN $\alpha$ R* ( $p = 0.02$ ), indicating that the highest expression of this gene was observed at the 200 ng/kg LPS treatment level. A significant change in the expression of *C3* and the *RPLPO* housekeeping gene across LPS doses was not observed.

The hepatic genes studied are either involved in LPS recognition, or in regulating the inflammatory response that occurs following LPS recognition. *CD14* for example, plays a key role in LPS recognition during bacterial infection. LPS ligation to *CD14*-TLR4 complex subsequently activates numerous cell types to secrete pro-inflammatory cytokines including IL-6. Recent studies performed with bovine MAC-T cells [15], and rat lung [16] and liver tissues [9] have shown that *CD14* expression levels were largely unaffected by LPS. An earlier rodent study however, reported up-regulation of *CD14* 3-hours, but not 6-hours post-challenge with 4  $\mu$ g/kg of LPS administered *i.v* [6]. Previously, our group demonstrated that ovine *CD14* gene expression increased significantly 2 hours, but not 5 hours after systemic challenge with 200 and 400 ng/kg of LPS [17]. In the present study, *CD14* gene expression was reduced at the 5-hour sampling time. These results and others suggest that LPS induces tissue-specific and temporal differences in *CD14* gene expression.

*NRAMP1* functions as a proton/divalent cation transporter in the membranes of the late endosomes/lysosomes, regulating cytoplasmic iron levels in macrophages, and plays a role in host innate immunity [18]. *NRAMP1* gene expression is dramatically increased in murine macrophages treated with LPS *in vitro* and *in vivo* [19], and its expression is both time- and dose-dependent [20]. A study by Wyllie *et al.* demonstrated using *NRAMP1* knockout mice that hepatic *NRAMP1* expression is important for inducing early phase Kupffer cell activation and hepatic inflammation [21]. The LPS dose-dependent down regulation of *NRAMP1* gene expression observed in the current study may be part of a regulatory mechanism

**Table 2: Primers, and gene accession number, annealing temperature, and product size for quantitative real-time PCR analysis**

Gene	Forward & Reverse primer	Accession no	(°C)	(bp)
<i>GAPDH</i>	CCTGGAGAAACCTGCCAAGT GCCAAATTCATTGTCGTACCA	<a href="#">TC186924</a>	58	226
<i>RPLPO</i>	CAACCCTGAAGTGCTTGACAT AGGCAGATGGATCAGCCA	<a href="#">TC204704</a>	62	220
<i>CD14</i>	TGAACATTGCCCAAGCACAC GCCGAGACTGGGATTGTCAG	<a href="#">AY289201</a>	58	101
<i>C3</i>	AGAAGCAGAAGCCTGATGGA CCTCGCAGATGTCTTTAGCC	<a href="#">AF038130</a>	60	150
<i>IFN<math>\alpha</math>R</i>	CGCATAAGAGCAGAAGAAGGA TGTTCCAGGGGAGAGATG	<a href="#">U65978</a>	60	150
<i>NRAMP1</i>	TTGGCACAGCTATTGCATTC TTCCGCAACCCGTAGTTATC	<a href="#">AF005380</a>	60	121
<i>SOD</i>	CGAGGCAAAGGGGAGATACAG TCTCCAAACTGATGGACGTG	<a href="#">M81129</a>	60	90
<i>MCP-1</i>	GGGTGCTCATTGACACCATC CATGGGAAGTCAAGGAGGAA	<a href="#">L32659</a>	60	126
<i>IGFBP3</i>	CAGAGCACAGACACCCAGAA TGCCCGTACTTATCCACACA	<a href="#">AF305199</a>	58	233

**Figure 1**

**Real-time PCR validation of the bovine microarray data.** Values are presented as a natural log<sub>e</sub> transformed mean  $\pm$  SE of 4 sheep per LPS dose group.

designed to control LPS-induced inflammation in the liver.

As a group of metal-containing enzymes, SODs have a vital anti-oxidant role conferred by their scavenging of the reactive oxygen species [22]. A previous study using rats demonstrated that SOD activity decreased in the liver during the acute phase of an *in vivo* LPS challenge and then increased during the recovery phase. Similar findings were reported with hepatocytes exposed to LPS *in vitro* in the same study [23]. A microarray study using liver tissue from rats challenged with LPS demonstrated induction of the SOD2 gene 24 hours post LPS challenge however, no assessment was made at earlier time points [9]. The dose-dependent decrease in SOD gene expression that was observed in the present study 4–5 hours post LPS chal-

lenge, combined with these previously reported studies, suggest that SOD gene expression varies temporally in the liver following LPS challenge.

IGFBPs regulate the bioactivity of mitogenic IGF-I and may also inhibit the growth of certain cell lines by an IGF-I receptor-independent pathway [24]. Priego *et al.* reported that LPS decreased the gene expression of IGFBP-3 in the rat liver following *in vivo* challenge [24]. Our results confirm their results using an ovine LPS challenge model.

MCP-1 is an important leukocyte chemoattractant that is involved in recruiting neutrophils and monocytes/macrophages during inflammation. Several studies have shown that LPS induces MCP-1 gene expression in various tissues

both *in vivo* and *in vitro* [25-27]. Two different LPS studies performed *in vivo* using the rat [9] and canine models [8] made no mention of hepatic MCP-1 induction using microarray analysis, although another chemokine, *MIP-1*, was reportedly induced 4 hours post LPS challenge in the canine model [8]. A recent study however, reported hepatic MCP-1 protein expression in mice challenged with LPS [28]. Our study demonstrates that LPS also induced hepatic MCP-1 gene expression in sheep.

All IFN subtypes are multifunctional cytokines that exhibit differential activities through a common receptor composed of the subunits IFN $\alpha$ R1 and IFN $\alpha$ R2 [29]. In this study we found that the expression of *IFN $\alpha$ R1* gene was induced after LPS treatment, but the highest expression was observed at the 200 ng/kg LPS treatment level. A study by Severa *et al.*, demonstrated that human mature dendritic cells modulate their sensitivity to IFN subtypes by differentially regulating the IFN $\alpha$ R subunits [30]. Future studies on *IFN $\alpha$ R* may help us understand its role during LPS-induced hepatic inflammation.

C3 is a key molecule in both the classical and alternative pathways of the complement cascade. The expression of the C3 gene appears to be dependent on LPS dose, sampling time, and cell type. LPS has been reported to induce C3 gene expression for example, in a human hepatoma cell line *in vitro* [31] and in human mononuclear phagocytes and human polymorphonuclear leukocytes *in vitro* [32,33]. Others however, have reported that C3 gene expression is decreased in monocytes stimulated with LPS [34], and that C3 protein expression follows a bell shaped curve when monocytes are stimulated *in vitro* with LPS between 0.1–100 ng/ml [35]. In the present study we report that C3 expression is suppressed in the ovine liver as determined by microarray analysis. Unfortunately, there was insufficient power to validate these results by qRT-PCR analysis.

## Conclusion

In this study, we have identified several potentially important genes that are differentially expressed during the ovine inflammatory response to LPS challenge using bovine cDNA microarray and qRT-PCR analyses. Their potential role in regulating inflammation warrants further investigation. A comparison of these results to those reported in the literatures suggest that hepatic gene expression in response to LPS is dependent on multiple factors including species, tissue, sampling time, the dose and type of LPS.

## Methods

### Liver biopsy trial

Twelve yearling Riduea-Arcott ewe lambs were arbitrarily assigned to three groups and challenged with LPS (0, 200

or 400 ng/kg) from *E. coli* serotype 0111:B4 (Sigma Chemical, St. Louis, MO) between 8 and 9 am. Liver biopsies (30–40 mg) were collected between 4 and 5 hours post-challenge, and tissues were immediately placed in RNAlater (Ambion, Austin, TX) and stored at -80°C until total RNA extraction was performed. The doses and biopsy sampling times were based on previous time trial experiment [17].

### RNA extraction and amplification

Total RNA was isolated with Trizol reagent (Invitrogen, Burlington, ON) [17], and amplified using the Genisphere's SenseAmp kit (Genisphere Inc. Hatfield, PA) according to the manufacturer's instructions. Briefly, 0.25 µg of total RNA was used to synthesize first strand cDNA using an oligo (dT) primer and random primer. First strand cDNA was purified then tailed with dTTP using Terminal Deoxynucleotidyl Transferase. The T4 template Oligo was annealed to the 3' end of the cDNA. Klenow enzyme fills in the 3' end of first strand cDNA to produce a double-stranded T7 promoter. Sense-strand RNA (sense RNA) copies of the original starting material were generated during *in vitro* transcription. Amplified sense RNA was quantified using Agilent 2000 Bioanalyzer.

### Construction of a bovine immune-endocrine cDNA microarray

A set of 109 immune, endocrine and inflammation-associated genes was selected for triplicate spotting onto Corning GAPS II slides using a VIRTEK Chip Maker Pro spotter (BioRad, Mississauga, Canada). Positive controls included 5 housekeeping genes ( *$\beta$ -actin*, *GAPDH*, *HPRT*, *PRLPO* and  *$\beta$ 2-microglobulin*), and a serial dilution of pooled bovine genomic DNA. Negative controls included a bacterial gene (VapA) and a partial plasmid sequence of *pACYC177*. All gene products were PCR amplified from either bacterial clones, or liver total RNA. The original clone sets and gene-specific primers were donated by Tao *et al.* [12].

### Microarray hybridization

For each sample, Alexa Fluor 555 or Alexa Fluor 647-labeled cDNAs were generated from 2–2.5 µg of SenseRNA using the SuperScript Indirect cDNA Labeling system (Invitrogen, Burlington, ON). Labelled control animal cDNA was then mixed with labeled cDNA of an animal from either the 200, or 400 ng/kg LPS dose groups, and then hybridized to the array for 18 h in a GeneTAC HybStation (Genomic Solutions, Ann Arbor, MI) using step-down temperatures from 65°C to 50°C in sealed chambers. Following hybridization, the station applies three washes, one with medium stringency buffer, one with high stringency buffer and one with post wash buffer (Genomic Solutions). Slides were finally rinsed briefly at room temperature in 2 × SSC and once in ddH<sub>2</sub>O. Washed

microarrays were dried by centrifugation at 1700 rpm for 2 min in a cushioned 50 ml tube. Dye swapping was performed on half of the samples to prevent dye bias. Dried Slides were scanned using GenePix™ 4000 (Axon Instruments Inc. Union City, USA). The images were analyzed and tabulated using GenePix Pro 3.0.

#### Microarray data analysis

Microarray data were normalized using LOWESS (Locally Regression and Smoothing Scatterplots) procedure of SAS. The program was obtained from Dr. P. Coussens (Department of Animal Science, Michigan State University). Normalized data were imported into Microsoft Excel, log transformed, and the median blank intensity on a microarray for each dye was subtracted from the respective normalized spot intensity values. These blank corrected values were then used to calculate a mean log expression difference between LPS-treated and control samples. The significance of the values was determined using a two-tailed Student's t-test. The antilog of the mean log expression difference for an individual gene on the array yielded the approximate fold change in expression between cDNA from the LPS-dose groups and control group.

#### Quantitative real-time PCR and analysis

To confirm gene expression differences observed from microarray results, qRT-PCR was performed on 9 genes. The primers for housekeeping gene, *GAPDH* and *RPLPO* were developed by Tellam [36]. Other primers were developed using Primer 3 software by our group (Table 2).

qRT-PCR was performed in triplicate for each sample on ABI 7000 Sequence detection system (Applied Biosystems, Streetsville, ON) using default two-step amplification procedures and 2 × SYBR Green Master Mix (Invitrogen, Burlington, ON) in a 25 µl reaction volume according to manufacture instructions. The conditions for the PCR reaction were: 50°C for 2 min, 95°C for 2 min followed by a maximum of 50 cycles of 95°C for 15 sec, annealing temperature for 30 sec and 72°C for 30 sec. The annealing temperatures for genes of interest are included in Table 2. The standard curve method was used to determine relative quantitation of mRNA abundance [37]. Statistical analysis was carried out on the qRT-PCR data using GLM of SAS (SAS 2002, SAS Institute Inc., Gary, NC). Orthogonal polynomial contrasts were performed on the least squares means to identify both linear and quadratic responses across dose. Residual plots were examined to assess homogeneity of variance.

#### Authors' contributions

HC carried out the microarray and real-time PCR experiment, participated in construction of the bovine immune-endocrine cDNA microarray and was responsible for man-

uscript preparation. LCK performed liver biopsy trial and total RNA isolation. QY carried out construction of the bovine immune-endocrine cDNA microarray. AR was responsible for collecting the liver biopsies. HJB was LCK's graduate co-supervisor. NAK facilitated the study, and participated in its design, coordination and analysis, and the liver biopsy sample collection, and helped to draft the manuscript. All authors read and approved the final manuscript.

#### Acknowledgements

We would like to acknowledge Margaret Howes in the Department of Molecular and Cellular Biology, University of Guelph, for printing the microarrays. We also thank Dr. Margaret Quinton in the Department of Animal and Poultry Science, University of Guelph, for her statistical help, and Dr. Bonnie Mallard for providing the set of clones for microarray construction. We thank Jeremy Mount, Carl McNicoll and the staff at Ponsonby Research Station for assistance with the liver biopsy sampling. This work was supported by research grants to Dr. Niel Karrow from the Natural Sciences and Engineering Research Council of Canada, and the Ontario Ministry of Agriculture and Food.

#### References

1. Cavaillon JM, Annane D: **Compartmentalization of the inflammatory response in sepsis and SIRS.** *Journal of Endotoxin Research* 2006, **12**:151-170.
2. Steiner AA, Ivanov AI, Serrats J, Hosokawa H, Phayre AN, Robbins JR, Roberts JL, Kobayashi S, Matsumura K, Sawchenko PE, Romanovsky AA: **Cellular and molecular bases of the initiation of fever.** *PLoS Biol* 2006, **4**:e284.
3. Karrow NA: **Activation of the hypothalamic-pituitary-adrenal axis and autonomic nervous system during inflammation and altered programming of the neuroendocrine-immune axis during fetal and neonatal development: lessons learned from the model inflammagen, lipopolysaccharide.** *Brain Behav Immun* 2006, **20**:144-158.
4. Munford RS: **Detoxifying endotoxin: time, place and person.** *J Endotoxin Res* 2005, **11**:69-84.
5. Shimada H, Hasegawa N, Koh H, Tasaka S, Shimizu M, Yamada W, Nishimura T, Amakawa K, Kohno M, Sawafuji M, Nakamura K, Fujishima S, Yamaguchi K, Ishizaka A: **Effects of initial passage of endotoxin through the liver on the extent of acute lung injury in a rat model.** *Shock* 2006, **26**:311-315.
6. Bulera SJ, Eddy SM, Ferguson E, Jatkoe TA, Reindel JF, Bleavins MR, De La Iglesia FA: **RNA expression in the early characterization of hepatotoxicants in Wistar rats by high-density DNA microarrays.** *Hepatology* 2001, **33**:1239-1258.
7. Liu S, Gallo DJ, Green AM, Williams DL, Gong X, Shapiro RA, Gambotto AA, Humphris EL, Vodovotz Y, Billiar TR: **Role of toll-like receptors in changes in gene expression and NF-κB activation in mouse hepatocytes stimulated with lipopolysaccharide.** *Infect Immun* 2002, **70**:3433-3442.
8. Higgins MA, Berridge BR, Mills BJ, Schultze AE, Gao H, Searfoss GH, Baker TK, Ryan TP: **Gene expression analysis of the acute phase response using a canine microarray.** *Tox Sci* 2003, **74**:470-484.
9. Xie W, Shao N, Ma X, Ling B, Wei Y, Ding Q, Yang G, Liu N, Wang H, Chen K: **Bacterial endotoxin lipopolysaccharide induces up-regulation of glyceraldehyde-3-phosphate dehydrogenase in rat liver and lungs.** *Life Sciences* 2006 in press.
10. Chitko-McKown CG, Fox JM, Miller LC, Heaton MP, Bono JL, Keen JE, Grosse WM, Laegreid WV: **Gene expression profiling of bovine macrophages in response to Escherichia coli O157:H7 lipopolysaccharide.** *Dev Comp Immunol* 2004, **28**:635-645.
11. Pareek R, Wellnitz O, Dorp RV, Burton J, Kerr D: **Immunorelevant gene expression in LPS challenged bovine mammary epithelial cells.** *J Appl Gene* 2005, **46**:71-177.

12. Tao W, Mallard B, Karrow N, Bridle B: **Construction and application of a bovine immune-endocrine cDNA microarray.** *Vet Immunol Immunopathol* 2004, **101**:1-17.
13. Donaldson L, Vuocolo T, Gray C, Strandberg Y, Reverter A, McWilliam S, Wang Y, Byrne K, Tellam R: **Construction and validation of a bovine innate immune microarray.** *BMC Genomics* 2005, **6**:135-156.
14. Goff LA, Bowers J, Schwalm J, Howerton K, Getts RC, Hart RP: **Evaluation of sense-strand mRNA amplification by comparative quantitative PCR.** *BMC Genomics* 2004, **5**:76-84.
15. Strandberg Y, Cray C, Vuocolo T, Donaldson L, Broadway M, Tellam R: **Lipopolysaccharide and lipoteichoic acid induce different innate immune response in bovine mammary epithelial cells.** *Cytokine* 31:72-86.
16. Oshikawa K, Sugiyama Y: **Regulation of toll-like receptor 2 and 4 gene expression in murine alveolar macrophages.** *Exp Lung Res* 2003, **29**:401-412.
17. Kabaroff LC, Rodriguez A, Quinton M, Boermans H, Karrow NA: **Assessment of the ovine acute phase response and hepatic gene expression in response to *Escherichia coli* endotoxin.** *Vet Immunol Immunopathol* 2006 in press.
18. Wyllie S, Seu P, Goss JA: **The natural resistance-associated macrophage protein 1 *Slc11a1* (formerly *Nramp1*) and iron metabolism in macrophages.** *Microbes Infect* 2002, **4**:351-359.
19. Govoni G, Vidal S, Cellier M, Lepage P, Malo D, Gros P: **Genomic structure, promoter sequence, and induction of expression of the mouse *Nramp1* gene in macrophages.** *Genomics* 1995, **27**:9-19.
20. Govoni G, Gauthier S, Billia F, Iscove NN, Gros P: **Cell-specific and inducible *Nramp1* gene expression in mouse macrophages in vitro and in vivo.** *J Leukoc Biol* 1997, **62**:277-286.
21. Wyllie S, Seu P, Gao FQ, Gros P, Goss JA: **Disruption of the *Nramp1* (also known as *Slc11a1*) gene in Kupffer cells attenuates early-phase, warm ischemia-reperfusion injury in the mouse liver.** *J Leukoc Biol* 2002, **72**:885-897.
22. Johnson F, Giulivi C: **Superoxide dismutases and their impact upon human health.** *Mol Aspects Med* 2005, **26**:340-352.
23. Portoles MT, Ainaga MJ, Pagani R: **The induction of lipid peroxidation by *E. coli* lipopolysaccharide on rat hepatocytes as an important factor in the etiology of endotoxic liver damage.** *Biochim Biophys Acta* 1993, **1158**:287-292.
24. Priego T, Ibanez de Caceres I, Martin AI, Villanua MA, Lopez-Calderon A: **Endotoxin decreases serum IGFBP-3 and liver IGFBP-3 mRNA: comparison between Lewis and Wistar rats.** *Mol Cell Endocrinol* 2003, **199**:23-28.
25. Türler A, Schwarz NT, Türler E, Kalff JC, Bauer AJ: **MCP-1 causes leukocyte recruitment and subsequently endotoxemic ileus in rat.** *Am J Physiol Gastrointest Liver Physiol* 2002, **282**:145-155.
26. Xia Y, Feng L, Yoshimura T, Wilson CB: **LPS-induced MCP-1, IL-1 beta, and TNF-alpha mRNA expression in isolated erythrocyte-perfused rat kidney.** *Am J Physiol Renal Physiol* 1993, **264**:F774-F780.
27. Bauermeister K, Burger M, Almanasreh N, Knopf HP, Schumann RR, Schollmeyer P, Dobos GJ: **Distinct regulation of IL-8 and MCP-1 by LPS and interferon-gamma-treated human peritoneal macrophages.** *Nephrol Dial Transplant* 1998, **13**:1412-1419.
28. Inoue K, Takano H, Shimada A, Morita T, Yanagisawa R, Sakurai M, Sato M, Yoshino S, Yoshikawa T: **Cytoprotection by interleukin-6 against liver injury induced by lipopolysaccharide.** *Int J Mol Med* 2005, **15**:221-224.
29. Jaitin DA, Roisman LC, Jaks E, Gavutis M, Piehler J, Van der Heyden J, Uze G, Schreiber G: **Inquiring into the differential action of interferons (IFNs): an IFN-alpha2 mutant with enhanced affinity to IFNAR1 is functionally similar to IFN-beta.** *Mol Cell Biol* 2006, **26**:1888-1897.
30. Severa M, Remoli ME, Giacomini E, Ragimbeau J, Lande R, Uze G, Pellegrini S, Coccia EM: **Differential responsiveness to IFN- $\alpha$  and IFN- $\beta$  of human mature DC through modulation of IFNAR expression.** *J Leukoc Biol* 2006, **79**:1286-1294.
31. Wright MS, Sund NJ, Abrahamsen TG: **Modulation of C3 gene expression in HepG2 human hepatoma cells.** *Immunol Lett* 2001, **76**:119-123.
32. Strunk RC, Whitehead AS, Cole FS: **Pretranslational regulation of the synthesis of the third component of complement in human mononuclear phagocytes by the lipid A portion of lipopolysaccharide.** *J Clin Invest* 1985, **76**:985-990.
33. Botto M, Lissandrini D, Sorio C, Walport MJ: **Biosynthesis and secretion of complement component (C3) by activated human polymorphonuclear leukocytes.** *J Immunol* 1992, **149**:1348-1355.
34. Shimizu H, Sakano T, Fujie A, Nishimura S, Ueda K: **Modulation of C2 and C3 gene expression of human peripheral blood monocytes by interleukin 1 beta, interferon gamma, tumor necrosis factor alpha and lipopolysaccharide.** *Experientia* 1992, **48**:148-150.
35. Hogasen AK, Hestdal K, Abrahamsen TG: **Granulocyte-macrophage colony-stimulating factor, but not macrophage colony-stimulating factor, suppresses basal and lipopolysaccharide-stimulated complement factor production in human monocytes.** *J Immunol* 1993, **151**:3215-3224.
36. Tellam R: **Construction and validation of a bovine innate immune microarray.** *BMC Genomics* 2005, **6**:135-156.
37. Larionov A, Krause A, Miller VV: **A standard curve based method for relative real time PCR data processing.** *BMC Bioinformatics* 2005, **6**:62-77.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
http://www.biomedcentral.com/info/publishing\_adv.asp

